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Patent Application No. 2002-70996

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[LIST OF DOCUMENTS SUBMITTED]

[TITLE OF DOCUMENT] Specification 1

[TITLE OF DOCUMENT] Abstract 1

[PROOF REQUIRED OR NOT] Required

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【Title of Document】 DESCRIPTION

【Title of the Invention】 Novel *N*-Acetylglucosaminyltransferase and Nucleic Acid Coding for the Same

【Claims】

【Claim 1】 A protein having the amino acid sequence shown in SEQ ID NO: 1 in SEQUENCE LISTING, or a protein having the same amino acid sequence as shown in SEQ ID NO:1 except that one or more amino acids are substituted or deleted, or that one or more amino acids are inserted or added, which has an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage.

【Claim 2】 The protein according to claim 1, which has the amino acid sequence shown in SEQ ID NO: 2 in SEQUENCE LISTING, or a protein having the same amino acid sequence as shown in SEQ ID NO:1 except that one or more amino acids are substituted or deleted, or that one or more amino acids are inserted or added.

【Claim 3】 The protein according to claim 1 or 2, wherein said protein has an amino acid sequence having a homology of not less than 70% to said amino acid sequence shown in SEQ ID NO:1 or 2.

【Claim 4】 The protein according to claim 3, wherein said protein has an amino acid sequence having a homology of not less than 90% to said amino acid sequence shown in SEQ ID NO:1 or 2.

【Claim 5】 The protein according to claim 4, wherein said protein has an amino acid sequence having the same amino acid sequence as shown in SEQ ID NO: 2 except that one or several amino acids are substituted or deleted, or that one or several amino acids are inserted or added.

【Claim 6】 The protein according to claim 5, which has the amino acid sequence shown in SEQ ID NO:2.

【Claim 7】 A protein comprising a region having the amino acid sequence recited in any one of claims 1 to 6, which has an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through

β1,3-linkage.

【Claim 8】 A nucleic acid coding for said protein according to any one of claims 1 to 7.

【Claim 9】 The nucleic acid according to claim 8, which hybridizes with the nucleic acid having the nucleotide sequence shown in SEQ ID NO:2 or 4 under stringent conditions.

【Claim 10】 The nucleic acid according to claim 9, which has the nucleotide sequence shown in SEQ ID NO:2 or 4.

【Claim 11】 A recombinant vector containing the nucleic acid according to any one of claims 8 to 10, which can express said nucleic acid in a host cell.

【Claim 12】 A cell into which said nucleic acid according to any one of claims 8 to 10 is introduced, which expresses said nucleic acid.

【Claim 13】 A nucleic acid for measurement of said nucleic acid according to any one of claims 8 to 10, which specifically hybridizes with said nucleic acid according to any one of claims 8 to 10.

【Claim 14】 The nucleic acid for measurement of nucleic acid, according to claim 13, which has a sequence complementary to a part of said nucleic acid of claim 10.

【Claim 15】 The nucleic acid for measurement of nucleic acid, according to claim 13 or 14, which is a probe or a primer.

【Claim 16】 The nucleic acid for measurement of nucleic acid, according to claim 15, which has not less than 15 bases.

【Detailed description of the invention】

【0001】

【Technical Field to Which the Invention Pertains】

The present invention relates to a novel enzyme having an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage, and to a nucleic acid coding for the same, as well as to nucleic acids for measuring the nucleic acid.

【0002】

【Prior Art】

Five types of enzymes are known, having an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage, which activity is involved in the synthesis of polylactosamine sugar chains (Togayachi, A. et al., J Biol Chem, 2001, 276, 22032-40; Shiraishi, N. et al., J Biol Chem, 2001, 276, 3498-507; Sasaki, K et al., Proc Natl Acad Sci U S A, 1997, 94, 14294-9). However, although the amount of polylactosamine on cell surfaces is increased by making the cells express the gene of the enzyme, some of the enzymes expressed have very low activities. Thus, although it is thought that the enzymes which produce polylactosamine have different characteristics, the characterization of the enzymes has not been sufficient. Therefore, to prepare or produce the polylactosamine sugar chain structure which requires the enzyme activity, it is necessary to chemically synthesize the structure, isolating the structure from a biological component or to synthesize the structure enzymatically using a tissue homogenate.

【0003】

It is known that sugar chain structures such as Lewis antigen exist on the sugar chain structures based on polylactosamine sugar chains (Kannagi R. Glycoconj J. 1997 Aug;14(5):577-84. Review; Nishihara S et al., J Biol Chem. 1994 Nov 18;269(46):29271-8). Similarly, it is said that the structures such as the lengths of polylactosamine sugar chains are involved in cellular immunity by NK cells or the like (Ohyama C et a., EMBO J. 1999 Mar 15;18(6):1516-25). Similarly, it is known that human stomach tissue is infected with *Helicobacter pylori* through a related sugar chain such as Lewis antigen (Wang G et al., Mol Microbiol. 2000 Jun;36(6):1187-96. Review; Falk PG et al., Proc Natl Acad Sci U S A. 1995 Feb 28;92(5):1515-9). Thus, if the gene of an enzyme having an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage can be cloned, and if the enzyme can be produced by a genetic engineering process using the gene, an antibody to the enzyme may also be produced. Therefore, these are useful for the diagnoses, therapies and prophylactics of cancers, immune diseases and infectious

diseases by *pylori*. However, the enzyme has not yet been purified or isolated, and there is no clue to the isolation of the enzyme and identification of the gene. As a result, an antibody to the enzyme has not been prepared.

【0004】

【Problems Which the Invention Tries to Solve】

Accordingly, an object of the present invention is to provide an enzyme having an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage, and a nucleic acid coding for the same. Another object of the present invention is to provide a recombinant vector which expresses the above-mentioned the nucleic acid in a host cell, to provide a cell in which the nucleic acid is introduced and which expresses the nucleic acid and the enzyme protein, and to provide the enzyme protein. Still another object of the present invention is to provide a nucleic acid for measurement of the above-mentioned nucleic acid according to the present invention, and to provide a method for producing the enzyme having the activity.

【0005】

【Means to Solve the Problems】

As mentioned above, since the enzyme of interest has not been isolated, it is impossible to know its partial amino acid sequence. In general, it is not easy to isolate and purify a protein contained in cells in a trace amount, and so isolation of the enzyme from cells, which has not been isolated so far, is expected not easy. The present inventors thought that if there is a homologous region among the nucleotide sequences of the various enzyme genes, which enzymes have relatively similar actions to that of the enzyme of interest, the gene of the enzyme of interest may also have the homologous sequence. After searching the nucleotide sequences of the known β1,3-*N*-acetylglucosaminyltransferase genes, β1,3-galactoslytransferase genes and β1,3-*N*-acetylgalactosaminyltransferase genes, a homologous region was discovered. Thus, based on the cloning by PCR using cDNA library, in which a primer was set in the homologous region, and after various considerations, the present inventors succeeded in

the cloning of the gene of the enzyme, and its nucleotide sequence and the deduced amino acid sequence were determined, thereby accomplishing the present invention.

【0006】

That is, the present invention provides a protein having the amino acid sequence shown in SEQ ID NO: 1 in SEQUENCE LISTING, or a protein having the same amino acid sequence as shown in SEQ ID NO:1 except that one or more amino acids are substituted or deleted, or that one or more amino acids are inserted or added, which has an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage. The present invention also provides a nucleic acid coding for the protein. The present invention further provides a recombinant vector containing the nucleic acid, which can express the nucleic acid in a host cell. The present invention still further provides a cell which is transformed by the recombinant vector, which expresses the nucleic acid. The present invention still further provides a nucleic acid for measurement of the nucleic acid, which specifically hybridizes with the nucleic acid.

【0007】

【Modes of the Invention】

The nucleic acid resulting from the removal of the initiation codon (ATG) from the nucleic acid encoding the protein of the present invention, which was cloned from a human antrum cDNA library by the method that will be described in detail in the Examples below, has the nucleotide sequence shown in SEQ ID NO: 4 in the SEQUENCE LISTING, and the deduced amino acid sequence encoded thereby is described below the nucleotide sequence. In SEQ ID NO:3, the amino acid sequence alone is shown. In the Examples below, the nucleic acid having the nucleotide sequence shown in SEQ ID NO:4 was incorporated into an expression vector, expressed in insect cells and it was confirmed that a protein having the above-mentioned enzyme activity was produced. By comparing the amino acid sequence shown in SEQ ID NO:3 and the amino acid sequence of a similar enzyme (concrete enzyme name: β3GnT2 : AB049584 which is the gene of β-1,3-*N*-acetylglucosaminyltransferase), it is thought that the region

with a relatively high homology, that is, the region from the 45th amino acid to the C-terminal of the amino acid sequence shown in SEQ ID NO:3 is the active domain of the enzyme, and that the above-mentioned enzyme activity is exhibited if this region consisting of 283 amino acids is contained. This 283 amino acids is shown in SEQ ID NO:1 and the nucleic acid encoding this, taken out from SEQ ID NO:4, is shown in SEQ ID NO:2.

【0008】

The protein (named "β3GnT-7") according to the present invention obtained in the Examples below is an enzyme having the following characteristics. Each of the characteristics as well as the methods for measuring them are described in detail in the Examples below.

Action: Transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc group or Galβ1-4GlcNAc group through β1,3-linkage. The reaction catalyzed by the enzyme, expressed in terms of reaction equation, is as follows: UDP-*N*-acetyl-D-glucosamine + β-D-galactosyl-1,4-D-glucosyl-R → UDP + *N*-acetyl-β-D-glucosaminyl-1,3-β-D-galactosyl-1,4-D-glucosyl-R, or UDP-*N*-acetyl-D-glucosamine + β-D-galactosyl-1,4-N-acetyl-D-glucosaminyl-R → UDP + *N*-acetyl-β-D-glucosaminyl-1,3-β-D-galactosyl-1,4-*N*-acetyl-D-glucosaminyl-R

Substrate Specificity: Galβ1-4Glc group or Galβ1-4GlcNAc group. In biological substances, these groups occurs abundantly as, for example, polylactosamine structures in glycoproteins (*O*-glycans and *N*-glycans) and glycolipids (lacto·neolacto series sugar chains and the like). Further, the Galβ1-4Glc groups or Galβ1-4GlcNAc groups contained in the basal structures of proteoglycans (keratan sulfate) and the like.

【0009】

In general, it is well-known in the art that there are cases wherein the physiological activity of a physiologically active protein such as an enzyme is retained even if the amino acid sequence of the protein is modified such that one or more amino acids in the amino acid sequence is substituted or deleted, or one or more amino acids are inserted or

added to the amino acid sequence. Therefore, a protein having the same amino acid sequence as shown in SEQ ID NO:1 or 3 except that one or more amino acids are substituted or deleted, or one or more amino acids are inserted or added, which protein has an activity to transfer *N*-acetylglucosamine to a non-reducing group of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage (the protein is hereinafter referred to as "modified protein" for convenience) is also within the scope of the present invention. The amino acid sequence of such a modified protein preferably has a homology of not less than 70%, preferably not less than 90%, still more preferably not less than 95% to the amino acid sequence shown in SEQ ID NO: 1 or 3. The homology of the nucleotide sequence may easily be calculated by using a well-known software such as FASTA, and such a software is available on the internet. Further, as the modified protein, one having the same amino acid sequence as shown in SEQ ID NO:1 or 3 except that one or several amino acids are substituted or deleted, or that one or several amino acids are inserted or added is especially preferred. Further, a protein containing the protein having the amino acid sequence shown in SEQ ID NO:1 or 3, or a modified protein thereof, which has an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage is also within the scope of the present invention. For example, in the Examples below, a nucleic acid encoding a membrane-bound type enzyme, in which a transmembrane region is ligated to the upstream of the amino acid sequence shown in SEQ ID NO:3 was also cloned, and such a membrane-bound type enzyme is also within the scope of the present invention.

【0010】

The present invention also provides nucleic acids coding for the amino acid sequence shown in SEQ ID NO:1 or 3 and nucleic acids coding for the amino acid sequences of the above-mentioned modified proteins. As the nucleic acid, DNA is preferred. As is well-known, due to degeneracy, there may be a plurality of codons each of which codes for the same single amino acid. However, as long as a nucleic acid codes for the above-described amino acid sequence, any nucleic acid having any nucleotide sequence is within the scope of the present invention. The nucleotide sequences of the cDNA

actually cloned in the Examples below are shown in SEQ ID NOs:2 and 4. Those nucleic acids which hybridize with the nucleic acid having the nucleotide sequence shown in SEQ ID NO:2 or 4 under stringent conditions (i.e., hybridization is performed at 50 to 65°C using a common hybridization solution such as 5 x Denhardt's reagent, 6 x SSC, 0.5% SDS or 0.1% SDS), and which code for the above-described modified proteins are within the scope of the present invention.

【0011】

The above-described nucleic acid according to the present invention can be prepared by the method described in detail in Example below. Alternatively, since the nucleotide sequence was clarified by the present invention, it can easily be prepared by using human antrum as the material and performing the well-known RT-PCR method. The above-described protein according to the present invention can also be easily prepared by, for example, incorporating the above-described nucleic acid according to the present invention into an expression vector, expressing the nucleic acid in a host cell, and purifying the produced protein.

【0012】

By inserting the above-described nucleic acid according to the present invention into a cloning site of an expression vector, a recombinant vector which can express the above-described nucleic acid in a host cell may be obtained. As the expression vector, various plasmid vectors and virus vectors for various host cells are well-known and commercially available. In the present invention, such a commercially available expression vector may preferably be employed. The methods for transforming or transducing host cells with such a recombinant vector are also well-known. The present invention also provides a cell into which the nucleic acid according to the present invention is introduced by transformation, transduction or transfection, which expresses the nucleic acid. The methods *per se* for introducing a foreign gene into a host cell are well-known, and the introduction of the foreign gene may easily be attained by, for example, using the above-mentioned recombinant vector. An example of the construction of a recombinant vector and a method for introducing the nucleic acid

according to the present invention into host cells using the recombinant vector are described in detail in the Examples below.

【0013】

Sugar chains may be bound to the protein according to the present invention, as long as the protein has the amino acid sequence described above and has the above-described enzyme activity. In other words, the term "protein" used herein also includes "glycoprotein".

【0014】

Since the nucleotide sequence of the cDNA of the novel enzyme according to the present invention was clarified by the present invention, nucleic acids for measurement according to the present invention (hereinafter referred to as simply "nucleic acid for measurement"), which specifically hybridize with the mRNA or the cDNA of the enzyme, were provided by the present invention. The term "specifically" herein means that the nucleic acid does not hybridize with other nucleic acids existing in the cells subjected to the test and hybridizes only with the above-described nucleic acid according to the present invention. Although it is preferred, in general, that the nucleic acid for measurement has a sequence homologous with a part of the nucleic acid having the nucleotide sequence shown in SEQ ID NO:2 or 4, mismatch of about 1 or 2 bases does not matter in many cases. The nucleic acid for measurement may be used as a probe or a primer in a nucleic acid-amplification method. To assure specificity, the number of bases in the nucleic acid for measurement is preferably not less than 15, more preferably not less than 18. In cases where the nucleic acid is used as a probe, the size is preferably not less than 15 bases, more preferably not less than 20 bases, and not more than the full length of the coding region. In cases where the nucleic acid is used as a primer, the size is preferably not less than 15 bases, more preferably not less than 18 bases, and less than 50 bases. The methods for measuring a test nucleic acid using a nucleic acid having a sequence complementary to a part of the test nucleic acid as a primer of a gene-amplification method such as PCR or as a probe are well-known, and the methods by which the mRNA of the enzyme according to the present invention was

measured by Northern blot or *in situ* hybridization are concretely described in detail in the Examples below. In the present specification, "measurement" includes detection, quantification and semi-quantification.

【0015】

The nucleic acid-amplification methods such as PCR are well-known in the art, and reagent kits and apparatuses therefor are commercially available, so that they may easily be carried out. By carrying out the nucleic acid-amplification method using a pair of the above-described nucleic acids for measurement according to the present invention as primers and using the test nucleic acid as a template, the test nucleic acid is amplified. In contrast, in cases where the test nucleic acid is not contained in the sample, the amplification does not occur. Therefore, by detecting the amplification product, whether the test nucleic acid exists in the sample or not may be determined. Detection of the amplification product may be carried out by a method in which the reaction solution after the amplification is subjected to electrophoresis, and the bands are stained with ethidium bromide or the like, or by a method in which the amplification product after electrophoresis is immobilized on a solid phase such as a nylon membrane, a labeled probe which specifically hybridizes with the test nucleic acid is hybridized with the test nucleic acid, and the label after washing is detected. Alternatively, the test nucleic acid in the sample may be quantified by the so called realtime detection PCR using a quencher fluorescent pigment and a reporter fluorescent pigment. Since the kits for realtime detection PCR are also commercially available, realtime detection PCR may also be carried out easily. The test nucleic acid may also be semi-quantified based on the intensity of the band resulted in electrophoresis. The test nucleic acid may be a mRNA or a cDNA reverse-transcribed from a mRNA. In cases where a mRNA is amplified as the test nucleic acid, NASBA method (3SR method, TMA method) using the above-described pair of primers may also be employed. NASBA method *per se* is well-known, and kits therefor are commercially available, so that NASBA method may easily be carried out using the above-described pair of primers.

【0016】

As the probe, labeled probe obtained by labeling the above-described nucleic acid for measurement with a fluorescent label, radioactive label, biotin label or the like may be used. Whether the test nucleic acid exists in the sample or not may be determined by immobilizing the test nucleic acid or amplification product thereof, hybridizing the labeled probe therewith, and measuring the label bound to the solid phase after washing. Alternatively, the nucleic acid for measurement is immobilized, the test nucleic acid is hybridized therewith, and the test nucleic acid bound to the solid phase is detected by a labeled probe or the like. In such a case, the nucleic acid for measurement immobilized on the solid phase is also called a probe.

【００１７】

By making the enzyme according to the present invention act on a glycoprotein, oligosaccharide or polysaccharide having (a) Galβ1-4Glc or Galβ1-4GlcNAc group(s), *N*-acetylglucosamine is bound to the non-reducing terminal(s) of the Galβ1-4Glc or Galβ1-4GlcNAc group(s) through β1,3-linkage. Thus, the enzyme according to the present invention may be used for modification of sugar chains of glycoproteins and for synthesis of saccharides. Further, by administering this enzyme as an immunogen to an animal, an antibody to this enzyme may be prepared, so that the enzyme may be measured by an immunoassay using the antibody. Therefore, the enzyme according to the present invention and the nucleic acid coding for the enzyme are useful for the preparation of such an immunogen. Such an antibody and the above-described nucleic acid for measurement are useful for the measurement of the enzyme in the body, and the measurement is useful for the diagnoses, therapies and preventions of cancers, immune diseases and infectious diseases by *pylori*.

【００１８】

【Examples】

The present invention will now be described by way of Examples. However, the present invention is not restricted to the Examples. In the following description, the nucleic acid having the nucleotide sequence shown in SEQ ID NO:5, for example, may also be referred to as "SEQ ID NO:5" for convenience.

【００１９】

1. Search of Gene Database and Determination of Nucleotide Sequence of β3GnT-7

Using analogous genes which are known β1,3-*N*-acetylglucosaminyltransferase genes, β1,3-galactosyltransferase genes and β1,3-*N*-acetylgalactosaminyltransferase gene, search of analogous genes was carried out on a gene database. The used sequences were β1,3-*N*-acetylglucosaminyltransferase genes with accession Nos.: AB049584, AB049585, AB049586 and AB045278; β1,3-galactosyltransferase genes of accession Nos. AF117222, Y15060, Y15014, AB026730, AF145784 and AF145784; and β1,3-*N*-acetylgalactosaminyltransferase gene with accession No. Y15062 (all of the accession Nos. are of GenBank). The search was carried out using a program tBlastn of BLAST, and all of the amino acid sequences corresponding to ORFs (Open Reading Frames) were included in the search.

【００２０】

As a result, EST sequences with GenBank Accession Nos. AK000770 and a human genomic sequence AC017104 were discovered. Thus, using AC017104, a library was screened.

【００２１】

The used sample was human antrum cDNA library prepared by a conventional method (Yuzuru Ikehara , Hisashi Narimatsu et al, Glycobiology vol. 9 no. 11 pp. 1213-1224, 1999). The screening was carried out by a usual nucleic acid probe method using a radio isotope. The concrete procedures were as follows:。

【００２２】

First, using the λ phage prepared from a human antrum cDNA library by a conventional method as templates, PCR was performed using as primers CB-635(5'-cagca gctgc tggcc tacga agac- 3') (nt6814-6837 in AC017104) and CB-638 (5'-gcaca tgccc agaaa gacgt cgtc-3') (nt7221-7245). The amplified DNA fragment having a size of about 430 bp was labeled with ^{32}P-dCTP using Multiple DNA labeling system produced by AMERSHAM.

【００２３】

Using this probe, single plaques which hybridized with this probe were picked up from the plaques of λ phage formed on *E. coli*. Existence of the target DNA region was confirmed by PCR using the above-mentioned primers CB635 and CB638. Since the phage obtained from the plaques, in which the insertion of the DNA fragment was confirmed was constructed by λ ZAP II vector (STRATAGENE) (Yuzuru Ikehara , Hisashi Narimatsu et al, Glycobiology vol. 9 no. 11 pp. 1213-1224, 1999), a cDNA clone inserted into pBluescript SK vector can be prepared (excision) by the method according to the manufacturer's instruction. The recombinant vector was prepared by this method, and a DNA was obtained from the obtained colony. The cDNA clone was then sequenced (SEQ ID NO:6).

【0024】

The SEQ ID NO:6 obtained by the above-described method corresponded to nt4828-7052 of AC017104 and lacked the 3' region of ORF. Therefore, the 3' region was cloned after amplification thereof by PCR using the cDNA, and was ligated. That is, a primer CB-625 (5'-cgttc ctggg cctca gtttc ctag-3') (nt7638-7661) corresponding to a region downstream of the termination codon was designed based on the sequence expected from AC017104 resulted from the search by computer, and using this primer in combination with the above-described CB635, a DNA fragment was obtained from the above-described human antrum cDNA library. The obtained DNA fragment was sequenced by a conventional method to obtain SEQ ID NO:7 (nt6814－7661 in AC017104) (hereinafter referred to as "SEQ ID NO:3"). By combining this with SEQ ID NO:2, a theoretical ORF of 978 bp (nt6466-7452 in AC017104) was obtained, and a sequence of 328 amino acids was deduced from this ORF, which was named β3GnT-7 (SEQ ID NO:8). It is known that glycosyltransferases are, in general, type 2 enzymes having one transmembrane segment. However, no hydrophobic region was found in the N-terminal region of this ORF sequence. Since it has been reported that β1,3-*N*-acetylglucosaminyltransferase activity is detected in human serum (Human Serum Contains *N*-Acetyllactosamine: β1,3-*N*-Acetylglucosaminyltransferase Activity. Hosomi, O., Takeya, A., and Kogure, T. J. Biochem.95, 1655-1659(1984)), the enzyme

encoded by this ORF was a secretory type enzyme having no transmembrane region.

【0025】

To show that the ORF having the sequence shown in SEQ ID NO:8 and the amino acid sequence encoded thereby actually exist and function (i.e., expressed), existence of the mRNA was checked by RT-PCR and confirmation of the PCR product by a restriction enzyme, and by direct sequencing (usual method) of the PCR product was carried out. As a result, it was confirmed that the above-described theoretical ORF surely existed and actually functioned.

【0026】

As mentioned above, although it is known that glycosyltransferases are, in general, type 2 enzymes having one transmembrane segment, there is no hydrophobic region in the N-terminal region of the amino acid sequence shown in SEQ ID NO:8, so that the enzyme was thought to be different from the usual glycosyltransferases. Thus, whether a splicing variant having a hydrophobic region (transmembrane segment) in the N-terminal region exists or not was checked by analyzing the nucleotide sequence in the 5' region (i.e., the N-terminal region of the amino acid sequence).

【0027】

First, using Human stomach Marathon-Ready cDNA (CLONETECH), 5'-RACE (Rapid amplification of cDNA ends) was performed. More particularly, using the AP1 primer included in Marathon cDNA (an adaptor AP1 was attached to the both ends of the DNA fragment, and an adaptor AP2 was attached to the both inner ends thereof) and a primer β3GnT-7RACE-5 (5'-GACCG ACTTG ACAAC CACCA GCA-3') corresponding to the found sequence region, PCR was performed (94°C for 60 seconds, 5 cycles of 94°C for 30 seconds-72°C for 3 minutes, 5 cycles of 94°C for 30 seconds-70°C for 3 minutes, and 25 cycles of 94°C-68°C for 3 minutes) was performed. The obtained DNA product was subjected to nested PCR (94°C for 60 seconds, 5 cycles of 94°C for 30 seconds-72°C for 3 minutes, 5 cycles of 94°C for 30 seconds-70°C for 3 minutes, and 15 cycles of 94°C-68°C for 3 minutes) using the AP2 primer included in Marathon cDNA and a primer β3GnT-7RACE-4 (5'- GTAGA CATCG CCCCT GCACT TCT-3'). The

obtained product was cloned into pGEMeasy (CLONETECH) and sequenced. As a result, the sequence upstream of the initiation codon of the earlier discovered SEQ ID NO:6 was obtained, and a transmembrane region was observed when deduced into amino acid sequence. However, although the 5' region of the nucleotide sequence in the vicinity of the transmembrane region was analyzed, the initiation codon of the ORF was not found.

【0028】

Thus, using GeneScan, HMMgene and the like which were softwares for analyzing gene regions, the translation region of the human genomic sequence AC017104 containing β3GnT-7 was analyzed. As a result, a first exon of 11 bases (about 3 amino acid) (nt4331-4341 of AC017104) containing the initiation codon was expected. Thus, using a primer corresponding to an upstream region of the initiation codon, PCR was performed in order to determine whether the expected region existed as a transcript.

【0029】

More particularly, PCR (30 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 60 seconds) was performed using as primers β3GnT-7RACE-8 (5'- GCCCA GAGCT GCGAG CCGCT-3') (nt4278-4300 in AC017104) and CB-638 (5'- GCACA TGCCC AGAAA GACGT CG-3')((nt7224-7245 in AC017104), as a template Human leukocyte Marathon-Ready cDNA, and LA-Taq (TaKaRa). As a result, an amplification product having a size of 1046 bases was obtained. This PCR product was purified and sequenced. It was proved, as expected from the above-described analysis of the translation region, the 3'-side (nt4341) in the first exon was ligated to nt6258 in a downstream region. By combining SEQ ID NOs: 6 and 7 and this result, the nucleotide sequence having 1206 bases shown in SEQ ID NO:5 and the amino acid sequence having 401 amino acids shown in SEQ ID NO:9 were obtained. The SEQ ID NO:5 was one in which the upstream regions of 219 bases (73 amino acids) (nt4331-4341 and nt6258-6465 in AC017104) were ligated to SEQ ID NO:8 (combination of SEQ ID NOs:6 and 7), and it was thought that nt4342-6257 was spliced. Since SEQ ID NO:5 contains a transmembrane segment (nt6265-6322 in AC017104), SEQ ID NO:5 and SEQ

ID NO:8 were thought to be the transmembrane type and secretory type having the same activity, respectively.

【0030】

2. Insertion of β3GnT-7 into Expression Vector

To examine the activity of β3GnT-7, β3GnT-7 was expressed in insect cells. Although it is thought that the activity may be confirmed enough by expressing the active region from the 119th amino acid to the C-terminal of SEQ ID NO:9, which region is relatively well conserved in the other genes of the same family, the active region from the 75th amino acid to the C-terminal of β3GnT-7 (SEQ ID NO:9) was expressed.

【0031】

The gene was incorporated into pFastBac of Gateway system from INVITROGEN, and then a Bacmid by Bac-to-Bac system from INVITROGEN was prepared.

【0032】

① Preparation of Entry Clone

PCR was performed using β3GnT-7S primer (5'-GGGGA CAAGT TTGTA CAAAA AAGCA GGCTT Cgcct ctcag gggcc ccagg cct-3') and β3GnT-7A primer (5'-GGGGA CCACT TTGTA CAAGA AAGCT GGGTC catgg gggct cagga gcaag tgcc-3') (the nucleotides shown in capital letters were the added sequence attL for GATEWAY hereinbelow described), and as a template the DNA of β3GnT-7 clone (the clone containing the theoretical ORF sequence) generated from the cDNA clone obtained by the screening and the DNA fragment obtained by PCR, to obtain an amplification product.

【0033】

This product was incorporated into pDONR201 by BP clonase reaction to prepare an "entry clone". The reaction was carried by incubating a mixture of 5 μl of the desired DNA fragment, 1 μl (150 ng) of pDONR201, 2 μl of reaction buffer and 2 μl of BP clonase mix at 25°C for 1 hour. After adding 1 μl of Proteinase K, the reaction mixture was left to stand at 37°C for 10 minutes, thereby terminating the reaction.

【0034】

Then the whole mixture (11 μl) was mixed with 100 μl of competent cells (*E. coli* DH5α), and after heat shock, the mixture was plated on an LB plate containing kanamycin. On the next day, colonies were collected, and existence of the desired DNA was directly confirmed by PCR. For double check, the nucleotide sequence of the DNA was confirmed, and vector (pDONR-β3Gn-T7) was extracted and purified.

【0035】

② Preparation of Expression Clone

The above-described entry clone has attL at the both ends of the inserted region, the attL being a recombination site used when λ phage is cut out from *E. coli*. By mixing the entry clone with LR clonase (a mixture of recombination enzymes Int, IHF and Xis of λ phage) and a destination vector, the inserted region is transferred to the destination vector so that an expression clone is prepared. These operations will now be described in detail.

【0036】

Firstly, a mixture of 1 μl of the entry clone, 0.5 μl (75 ng) of pFBIF, 2 μl of LR reaction buffer, 4.5 μl of TE and 2 μl of LR clonase mix were allowed to react at 25°C for 1 hour, and then 1 μl of Proteinase K was added, followed by incubation at 37°C for 10 minutes, thereby terminating the reaction (by this recombination reaction, pFBIF-β3Gn-T7 is generated). The pFBIF was one obtained by inserting Igκ signal sequence (MHFQVQIFSFLLISASVIMSRG) and FLAG peptide (DYKDDDDK) for purification. The Igκ signal sequence was inserted in order to change the expressed protein to a secretory protein, and the FLAG peptide was inserted for purification. The DNA fragment obtained by PCR using as a template OT3 (5'-gatca tgcat tttca agtgc agatt ttcag cttcc tgcta atcag tgcct cagtc ataat gtcac gtgga gatta caagg acgac gatga caag-3'), and using primers OT20 (5'- cgggatccat gcattttcaa gtgcag-3') and OT21 (5'-ggaat tcttgt catcg tcgtc cttg-3') was inserted using *Bam* HI and *Eco* RI. Further, to insert the Gateway sequence, Conversion cassette was inserted using Gateway Vector Conversion System (INVITROGEN).

【0037】

Then the whole mixture (11 μl) was mixed with 100 μl of competent cells (*E. coli* DH5α), and after heat shock, the mixture was plated on an LB plate containing ampicillin. On the next day, colonies were collected, and existence of the desired DNA was directly confirmed by PCR, followed by extraction and purification of the vector (pFBIF-β3Gn-T7).

【0038】

③ Preparation of Bacmid by Bac-to-Bac Systema

Using Bac-to-Bac system (INVITROGEN), recombination was carried out between the above-described pFBIF- and pFastBac, and G10 and other sequences were inserted into a Bacmid which was able to replicate in insect cells. With this system, the desired gene is incorporated into the Bacmid by the recombinant protein produced by a helper plasmid, only by incorporating pFastBac into which the desired gene was inserted, using the recombination site of Tn7 into an *E. coli* (DH10BAC) containing the Bacmid. The Bacmid contains *lac*Z gene, so that classical selection based on the color, that is, blue (no insertion) or white (with insertion), of the colony can be attained.

【0039】

That is, the above-described purified vector (pFBIH-β3GnT-7) was mixed with 50 μl of competent cells (*E. coli* DH10BAC), and after heat shock, the mixture was plated on an LB plate containing kanamycin, gentamycin, tetracycline, Bluo-gal and IPTG. On the next day, white single colony was further cultured and Bacmid was collected.

【0040】

3. Introduction of Bacmid into Insect Cells

After confirming that the desired sequence was inserted into the Bacmid obtained from the white colony, the Bacmid was introduced into insect cells Sf21 (commercially available from INVITROGEN). That is, to a 35 mm Petri dish, Sf21 cells in an amount of 9×10^5 cells/2 ml (Sf-900SFM (INVITROGEN) containing an antibiotic) were added, and the cells were cultured at 27°C for 1 hour to adhere the cells. (Solution A): To 5 μl of the purified Bacmid DNA, 100 μl of Sf-900SFM (INVITROGEN) not containing an antibiotic was added. (Solution B): To 6 μl of CellFECTIN Reagent (INVITROGEN),

100 µl of Sf-900SFM (INVITROGEN) not containing an antibiotic was added. Solution A and Solution B were then gently mixed and the mixture was incubated for 15 to 45 minutes at room temperature. After confirming that the cells adhered, the culture medium was aspirated and 2 ml of Sf-900SFM (INVITROGEN) not containing an antibiotic was added. To a solution (lipid-DNA complexes) prepared by mixing Solution A and Solution B, 800 µl of Sf900II not containing an antibiotic was added and the resultant was gently mixed. The culture medium was aspirated, and diluted lipid-DNA complexes solution was added to the cells, followed by incubating the cells at 27°C for 5 hours. Thereafter, transfection mixture was removed and 2 ml of culture medium Sf-900SFM (INVITROGEN) containing an antibiotic was added, followed by incubating the resultant at 27°C for 72 hours. Seventy two hours after the transfection, the cells were peeled off by pipetting, and the cells and the culture medium were collected. The cells and the culture medium were centrifuged at 3000 rpm for 10 minutes, and the obtained supernatant was stored in a separate tube (this supernatant is the primary virus solution).

【0041】

To a T75 culture flask, Sf21 cells in an amount of 1×10^7 cells/20 ml of Sf-900SFM (INVITROGEN) (containing an antibiotic) were placed, and the resultant was incubated at 27°C for 1 hour. After the cells adhered, 800 µl of the primary virus was added and the resultant was cultured at 27°C for 48 hours. Forty eight hours later, the cells were peeled off by pipetting and the cells and the culture medium were collected. The cells and the culture medium were centrifuged at 3000 rpm for 10 minutes, and the obtained supernatant was stored in a separate tube (this supernatant was used as the secondary virus solution).

【0042】

Further, to a T75 culture flask, Sf21 cells in an amount of 1×10^7 cells/20 ml of Sf-900SFM (INVITROGEN) (containing an antibiotic) were placed, and the resultant was incubated at 27°C for 1 hour. After the cells adhered, 1000 µl of the secondary virus solution was added and the resultant was cultured at 27°C for 72 to 96 hours.

After the culturing, the cells were peeled off by pipetting and the cells and the culture medium were collected. The cells and the culture medium were centrifuged at 3000 rpm for 10 minutes, and the obtained supernatant was stored in a separate tube (this supernatant was used as the tertiary virus solution). Further, to a 100 ml spinner flask, 100 ml of Sf21 cells at a population of 6×10^5 cells/ml was placed, and 1 ml of the tertiary virus solution was added, followed by culturing the cells at 27°C for about 96 hours. After the culturing, the cells and the culture medium were collected. The cells and the culture medium were centrifuged at 3000 rpm for 10 minutes, and the obtained supernatant was stored in a separate tube (this supernatant was used as the quaternary virus solution).

【0043】

The primary to tertiary cell pellets were sonicated (sonication buffer: 20mM HEPES pH7.5, 2 % Triton X-100 (trademark)) and the crude cell extract was 20-fold diluted with H_2O. The resultant was subjected to SDS-PAGE and then to Western blotting using anti-FLAG M2-peroxidase (A-8592, SIGMA) in order to confirm the expression of β3Gn-T7 protein. As a result, a plurality of broad bands (thought to be due to differences in post-translational modifications by sugar chains or the like) centering at the position of about 38-40 kDa were detected, so that the expression was confirmed.

【0044】

4. Resin Purification of β3Gn-T7

To 10 ml of the supernatant of FLAG-β3Gn-T7 of the quaternary infection, NaN_3 (0.05 %), NaCl (150 mM), $CaCl_2$ (2 mM), and anti-M1 resin (SIGMA) (50 μl) were added and the resulting mixture was stirred overnight at 4°C. On the next day, the mixture was centrifuged (3000 rpm for 5 minutes, at 4°C) and the pellet was collected. To the pellet, 900 μl of 2 mM $CaCl_2$·TBS was added and the resultant was centrifuged again (2000 rpm for 5 minutes, at 4°C), and the pellet was suspended in 200 μl of 1 mM $CaCl_2$·TBS to obtain a sample (β3GnT-7 enzyme solution) for the measurement of activity.

【0045】

5. Search of Acceptor Substrate of β3Gn-T7

As a result of molecular evolutionary analysis comparing β3Gn-T7 with β1,3-*N*-acetylglucosaminyltransferases and β1,3-galactosyltransferases, β3Gn-T7 was classified into β1,3-*N*-acetylglucosaminyltransferases. Thus, firstly, analysis was performed using UDP-GlcNAc as the donor substrate.

【0046】

Using the following reaction systems, the acceptor substrate was searched. As the "acceptor substrate" in the reaction solution described below, each of the following was used and whether each of them functioned as the acceptor or not was investigated: *p*Np-α-Glc, *p*Np-β-Glc, *p*Np-α-GlcNAc, *p*Np-β-GlcNAc, *p*Np-α-Gal, *p*Np-β-Gal, *p*Np-α-GalNAc, Bz-α-GalNAc, *p*Np-α-Xyl, *p*Np-β-Xyl, *p*Np-α-Fuc, Bz-α-Man, Bz-α-ManNAc, LacCer, GalCer typel and Bz-β-lactoside (all of them are from SIGMA) and Galβ1-4GlcNAc-α-*p*Np (TRONTO RESEARCH CHEMICAL).

【0047】

The reaction solution (the numbers in the parentheses indicate the final concentrations) contained acceptor substrate (10 nmol), sodium cacodylate buffer (pH7.2) (50mM), Triton CF-54 (trademark) (0.4%), $MnCl_2$ (10 mM), UDP-GlcNAC (480 μM) and UDP-[^{14}C]GlcNAC (175 nCi) and CDP-colline (5 mM), to which 10 μl of the β3Gn-T7 enzyme solution and H_2O were added to attain a final volume of 25 μl.

【0048】

The reaction mixture was allowed to react at 37°C for 5 hours, and after completion of the reaction, 200 μl of 0.1 M KCl was added, followed by light centrifugation and collection of the supernatant. The supernatant was passed through Sep-Pak plus C18 Cartridge (WATERS) equilibrated by washing once with 10 ml of methanol and then twice with 10 ml of H_2O, so as to adsorb the substrate and the product in the supernatant on the cartridge. After washing the cartridge twice with 10 ml of H_2O, the adsorbed substrate and the product were eluted with 5 ml of methanol. The eluted solution was evaporated to dryness by blowing nitrogen gas while heating the solution with a heat block at 40°C. To the resultant, 20 μl of methanol was added, and the resulting mixture

was plotted on a TLC plate (HPTLC plate Silica gel 60: MERCK), and developed using a developing solvent having the composition of chloroform:methanol:water (containing 0.2% $CaCl_2$) = 65:35:8. After developing the mixture up to 5 mm from the top end of the TLC plate, the plate was dried and the intensity of the radioactivity taken in the product was measured using Bio Image Analyzer FLA3000 (FUJI PHOTO FILM).

【0049】

As a result, it was proved that β3GnT-7 is a β1,3-*N*-acetylglucosaminyltrasferase having an activity to transfer GlcNAc to Bz-β-lactoside and Galβ1-4Glc(NAc)-α-*p*Np, that is, an enzyme which transfers GlcNAc to the galactose at the non-reducing terminal of Galβ1-4Glc(NAc)-R.

【0050】

6. Analysis of Tissue-specific Expression of β3GnT-7

The expression of the gene in tissues and in cell lines was examined by Real Time PCR method (Gibson, U. E., Heid, C. A., and Williams, P. M. (1996) Genome Res 6, 995-1001). Human tissue cDNAs used as materials were the Marathon cDNAs. From the various cell lines, total RNAs were extracted by a conventional method and the cDNAs were synthesized. For obtaining the calibration curve of β3GnT-7, a plasmid containing β3GnT-7 gene inserted in pDONR^{TM}201 vector DNA was used. As a control for the endogenous expression, constantly expressed human glyceraldehyde-3-phosphate dehydrogenase (GAPDH)) was used. For obtaining the calibration curve of GAPDH, a plasmid containing the GAPDH gene in pCR2.1 (INVITROGEN) was used. As the primer set and probe for β3GnT-7, the following were used: RT-β3GnT-7-F2; 5'-TTCCTCAAGTGGCTGGACATC-3', RT-β3GnT-7-R2;5'-GCCGGTCAGCCAGAAATTC-3', probe;5'- Fam ACTGCCCCCACGTCCCCTTCA -MGB-3'. As the primer set and probe for GAPDH, a kit (Pre-Developed TaqMan® Assay Reagents Endogenous Human GAPDH (APPLIED BIOSYSTEMS) was used. The PCR was performed using TaqMan Universal PCR Master Mix (APPLIED BIOSYSTEMS) under the conditions of 50°C for 2 minutes, then at 95°C for 10 minutes, and repeating 50 cycles of 95°C for 15 seconds-60°C for 1 minute.

The quantitation of the PCR product was carried out using ABI PRIAM7700 Sequence Detection System (APPLIED BIOSYSTEMS). The expression amount of G11 was normalized by dividing the amount by the amount of the transcription product of the constantly expressed GAPDH. The results for the human tissues are summarized in Table 1, and the results for the cell lines are summarized in Table 2.

【0051】

【Table 1】

Table 1

Tissue	β3GnT-7/GAPDH
brain	0.01045
cerebral cortex	0.04522
cerebellum	0.02345
fetal brain	0.02030
bone marrow	0.01462
thyroid	0.04084
thymus	0.01274
spleen	0.10108
leukocyte	0.07876
heart	0.00956
skeletal muscle	0.00071
lung	0.12146
liver	0.02299
esophagus	0.00605
stomach	0.26922
small intestine	0.09333
colon	0.07630
pancreas	0.27317
kidney	0.01161
adrenal	0.15069
mammary gland	0.02560
uterus	0.07747
placenta	0.18763
ovary	0.11465
testis	0.05323

【0052】

The tissues in which β3GnT-7 was highly expressed were pancreas, stomach, placenta and adrenal, and the tissues in which β3GnT-7 was moderately expressed were colon, leukocyte, lung, ovary, small intestine, spleen, testis, uterus and cerebral cortex. In the

tissues other than these tissues, the expression amount was relatively low.

【0053】

【Table 2】

Table 2

Cell (origin)	β3GnT-7/GAPDH
GOTO (neuroblastoma)	0.00012
SCCH-26 (neuroblastoma)	0.00137
T98G (glioblastoma)	0.00032
U251 (glioblastoma)	0.00023
Leukemia (premyeloblastic leukemia)	0.35660
Melanoma (skin)	0.01255
HL-60 (premyeloblastic leukemia)	0.17663
K562 (leukemia)	0.00038
U937 (monocyte)	0.01617
Daudi (B cell (Burkitt's))	0.00437
PC-1 (lung)	0.00000
EBC-1 (lung)	0.00121
PC-7 (lung)	0.00017
HepG2 (liver)	0.01199
A431 (esophagus)	0.01031
MKN45 (stomach)	0.00027
KATOIII (stomach)	0.03964
HSC43 (stomach)	0.00031
Colo205 (colon)	0.00278
HCT15 (colon)	0.00193
LSC (colon)	0.00003
LSB (colon)	0.00128
SW480 (colon)	0.00045
SW1116 (colon)	0.13076
Capan-2 (pancreas)	0.03664
PA-1 (uterus)	0.00290

【0054】

Expression of β3GnT-7 in cell lines was lower than that in normal tissues. In HL60 cells originated from premyeloblastic leukemia and in SW1116 cells originated from colon, the expression level was high.

【0055】

It was easily thought that the expression amount of β3GnT-7 is changed when the degree of differentiation is changed by cancerization or the like, so that there is a possibility that measurement of the expression amount of β3GnT-7 may be used for

diagnoses of diseases. Further, as described above, there is a possibility that there are two initiation sites in β3GnT-7, so that there is a possibility that by measuring the change of the splicing variants, the state of differentiation and pathological change of the cells may be measured.

【0056】

【Effect of the Invention】

The present invention first provides an enzyme, which has an activity to transfer N-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage, and also a nucleic acid coding for the enzyme. The enzyme according to the present invention can be used for modifications of the sugar chains of glycoproteins and/or glycolipids, and also for syntheses of sugars. In addition, the present invention first provides a nucleic acid for measurement of the nucleic acid coding for the enzyme.

OCT 18 2006
PATENT & TRADEMARK OFFICE

【００５７】

【Sequence Listing】

SEQUENCE LISTING

<110> NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY
JAPAN GENOME SOLUTIONS INC.

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Ala Ser Gln Gly Pro Gln Ala Trp Asp Val Thr Thr Thr Asn Cys Ser
1 5 10 15
GCC AAT ATC AAC TTG ACC CAC CAG CCC TGG TTC CAG GTC CTG GAG CCG 96
Ala Asn Ile Asn Leu Thr His Gln Pro Trp Phe Gln Val Leu Glu Pro
20 25 30
CAG TTC CGG CAG TTT CTC TTC TAC CGC CAC TGC CGC TAC TTC CCC ATG 144
Gln Phe Arg Gln Phe Leu Phe Tyr Arg His Cys Arg Tyr Phe Pro Met
35 40 45

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CTG CTG AAC CAC CCG GAG AAG TGC AGG GGC GAT GTC TAC CTG CTG GTG    192
Leu Leu Asn His Pro Glu Lys Cys Arg Gly Asp Val Tyr Leu Leu Val
    50                  55                  60
GTT GTC AAG TCG GTC ATC ACG CAG CAC GAC CGC CGC GAG GCC ATC CGC    240
Val Val Lys Ser Val Ile Thr Gln His Asp Arg Arg Glu Ala Ile Arg
65                  70                  75                  80
CAG ACC TGG GGC CGC GAG CGG CAG TCC GCG GGT GGG GGC CGA GGC GCC    288
Gln Thr Trp Gly Arg Glu Arg Gln Ser Ala Gly Gly Gly Arg Gly Ala
                85                  90                  95
GTG CGC ACC CTC TTC CTG CTG GGC ACG GCC TCC AAG CAG GAG GAG CGC    336
Val Arg Thr Leu Phe Leu Leu Gly Thr Ala Ser Lys Gln Glu Glu Arg
            100                 105                 110
ACG CAC TAC CAG CAG CTG CTG GCC TAC GAA GAC CGC CTC TAC GGC GAC    384
Thr His Tyr Gln Gln Leu Leu Ala Tyr Glu Asp Arg Leu Tyr Gly Asp
        115                 120                 125
ATC CTG CAG TGG GGC TTT CTC GAC ACC TTC TTC AAC CTG ACC CTC AAG    432
Ile Leu Gln Trp Gly Phe Leu Asp Thr Phe Phe Asn Leu Thr Leu Lys
    130                 135                 140
GAG ATC CAC TTC CTC AAG TGG CTG GAC ATC TAC TGC CCC CAC GTC CCC    480
Glu Ile His Phe Leu Lys Trp Leu Asp Ile Tyr Cys Pro His Val Pro
145                 150                 155                 160
TTC ATT TTC AAA GGC GAC GAT GAC GTC TTC GTC AAC CCC ACC AAC CTG    528
Phe Ile Phe Lys Gly Asp Asp Asp Val Phe Val Asn Pro Thr Asn Leu
                165                 170                 175
CTA GAA TTT CTG GCT GAC CGG CAG CCA CAG GAA AAC CTG TTC GTG GGC    576
Leu Glu Phe Leu Ala Asp Arg Gln Pro Gln Glu Asn Leu Phe Val Gly
            180                 185                 190
GAT GTC CTG CAG CAC GCT CGG CCC ATT CGC AGG AAA GAC AAC AAA TAC    624
Asp Val Leu Gln His Ala Arg Pro Ile Arg Arg Lys Asp Asn Lys Tyr
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        195                 200                 205
TAC ATC CCG GGG GCC CTG TAC GGC AAG GCC AGC TAT CCG CCG TAT GCA    672
Tyr Ile Pro Gly Ala Leu Tyr Gly Lys Ala Ser Tyr Pro Pro Tyr Ala
    210                 215                 220
GGC GGC GGT GGC TTC CTC ATG GCC GGC AGC CTG GCC CGG CGC CTG CAC    720
Gly Gly Gly Gly Phe Leu Met Ala Gly Ser Leu Ala Arg Arg Leu His
225                 230                 235                 240
CAT GCC TGC GAC ACC CTG GAG CTC TAC CCG ATC GAC GAC GTC TTT CTG    768
His Ala Cys Asp Thr Leu Glu Leu Tyr Pro Ile Asp Asp Val Phe Leu
                245                 250                 255
GGC ATG TGC CTG GAG GTG CTG GGC GTG CAG CCC ACG GCC CAC GAG GGC    816
Gly Met Cys Leu Glu Val Leu Gly Val Gln Pro Thr Ala His Glu Gly
            260                 265                 270
TTC AAG ACT TTC GGC ATC TCC CGG AAC CGC AAC AGC CGC ATG AAC AAG    864
Phe Lys Thr Phe Gly Ile Ser Arg Asn Arg Asn Ser Arg Met Asn Lys
        275                 280                 285
GAG CCG TGC TTT TTC CGC GCC ATG CTC GTG GTG CAC AAG CTG CTG CCC    912
Glu Pro Cys Phe Phe Arg Ala Met Leu Val Val His Lys Leu Leu Pro
    290                 295                 300
CCT GAG CTG CTC GCC ATG TGG GGG CTG GTG CAC AGC AAT CTC ACC TGC    960
Pro Glu Leu Leu Ala Met Trp Gly Leu Val His Ser Asn Leu Thr Cys
305                 310                 315                 320
TCC CGC AAG CTC CAG GTG CTC                                        981
Ser Arg Lys Leu Gln Val Leu
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<210> 5

<211> 1206

<212> DNA

<213> Homo sapiens

<400> 5

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gcc ctg ctc gtg gcc gtg acg gtg ttc caa cgc agt ctc acc cct ggt 96
Ala Leu Leu Val Ala Val Thr Val Phe Gln Arg Ser Leu Thr Pro Gly
20 25 30

cag ttt ctg cag gag cct ccg cca ccc acc ctg gag cca cag aag gcc 144
Gln Phe Leu Gln Glu Pro Pro Pro Thr Leu Glu Pro Gln Lys Ala
35 40 45

cag aag cca aat gga cag ctg gtg aac ccc aac aac ttc tgg aag aac 192
Gln Lys Pro Asn Gly Gln Leu Val Asn Pro Asn Asn Phe Trp Lys Asn
50 55 60

ccg aaa gat gtg gct gcg ccc acg ccc atg gcc tct cag ggg ccc cag 240
Pro Lys Asp Val Ala Ala Pro Thr Pro Met Ala Ser Gln Gly Pro Gln
65 70 75 80

gcc tgg gac gtg acc acc act aac tgc tca gcc aat atc aac ttg acc 288
Ala Trp Asp Val Thr Thr Thr Asn Cys Ser Ala Asn Ile Asn Leu Thr
85 90 95

cac cag ccc tgg ttc cag gtc ctg gag ccg cag ttc cgg cag ttt ctc 336
His Gln Pro Trp Phe Gln Val Leu Glu Pro Gln Phe Arg Gln Phe Leu
100 105 110

ttc tac cgc cac tgc cgc tac ttc ccc atg ctg ctg aac cac ccg gag 384
Phe Tyr Arg His Cys Arg Tyr Phe Pro Met Leu Leu Asn His Pro Glu
115 120 125

aag tgc agg ggc gat gtc tac ctg ctg gtg gtt gtc aag tcg gtc atc 432
Lys Cys Arg Gly Asp Val Tyr Leu Leu Val Val Val Lys Ser Val Ile
130 135 140

acg cag cac gac cgc cgc gag gcc atc cgc cag acc tgg ggc cgc gag 480
Thr Gln His Asp Arg Arg Glu Ala Ile Arg Gln Thr Trp Gly Arg Glu
145 150 155 160
cgg cag tcc gcg ggt ggg ggc cga ggc gcc gtg cgc acc ctc ttc ctg 528
Arg Gln Ser Ala Gly Gly Gly Arg Gly Ala Val Arg Thr Leu Phe Leu
165 170 175
ctg ggc acg gcc tcc aag cag gag gag cgc acg cac tac cag cag ctg 576
Leu Gly Thr Ala Ser Lys Gln Glu Glu Arg Thr His Tyr Gln Gln Leu
180 185 190
ctg gcc tac gaa gac cgc ctc tac ggc gac atc ctg cag tgg ggc ttt 624
Leu Ala Tyr Glu Asp Arg Leu Tyr Gly Asp Ile Leu Gln Trp Gly Phe
195 200 205
ctc gac acc ttc ttc aac ctg acc ctc aag gag atc cac ttc ctc aag 672
Leu Asp Thr Phe Phe Asn Leu Thr Leu Lys Glu Ile His Phe Leu Lys
210 215 220
tgg ctg gac atc tac tgc ccc cac gtc ccc ttc att ttc aaa ggc gac 720
Trp Leu Asp Ile Tyr Cys Pro His Val Pro Phe Ile Phe Lys Gly Asp
225 230 235 240
gat gac gtc ttc gtc aac ccc acc aac ctg cta gaa ttt ctg gct gac 768
Asp Asp Val Phe Val Asn Pro Thr Asn Leu Leu Glu Phe Leu Ala Asp
245 250 255
cgg cag cca cag gaa aac ctg ttc gtg ggc gat gtc ctg cag cac gct 816
Arg Gln Pro Gln Glu Asn Leu Phe Val Gly Asp Val Leu Gln His Ala
260 265 270
cgg ccc att cgc agg aaa gac aac aaa tac tac atc ccg ggg gcc ctg 864
Arg Pro Ile Arg Arg Lys Asp Asn Lys Tyr Tyr Ile Pro Gly Ala Leu
275 280 285
tac ggc aag gcc agc tat ccg ccg tat gca ggc ggc ggt ggc ttc ctc 912
Tyr Gly Lys Ala Ser Tyr Pro Pro Tyr Ala Gly Gly Gly Gly Phe Leu

290 295 300

atg gcc ggc agc ctg gcc cgg cgc ctg cac cat gcc tgc gac acc ctg 960
Met Ala Gly Ser Leu Ala Arg Arg Leu His His Ala Cys Asp Thr Leu
305 310 315 320

gag ctc tac ccg atc gac gac gtc ttt ctg ggc atg tgc ctg gag gtg 1008
Glu Leu Tyr Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu Glu Val
325 330 335

ctg ggc gtg cag ccc acg gcc cac gag ggc ttc aag act ttc ggc atc 1056
Leu Gly Val Gln Pro Thr Ala His Glu Gly Phe Lys Thr Phe Gly Ile
340 345 350

tcc cgg aac cgc aac agc cgc atg aac aag gag ccg tgc ttt ttc cgc 1104
Ser Arg Asn Arg Asn Ser Arg Met Asn Lys Glu Pro Cys Phe Phe Arg
355 360 365

gcc atg ctc gtg gtg cac aag ctg ctg ccc cct gag ctg ctc gcc atg 1152
Ala Met Leu Val Val His Lys Leu Leu Pro Pro Glu Leu Leu Ala Met
370 375 380

tgg ggg ctg gtg cac agc aat ctc acc tgc tcc cgc aag ctc cag gtg 1200
Trp Gly Leu Val His Ser Asn Leu Thr Cys Ser Arg Lys Leu Gln Val
385 390 395 400

ctc tga 1206
Leu

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<210> 6
<211> 2228
<212> DNA
<213> Homo sapiens
<400> 6

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gcggcttccc tggaacccct agggctggca gggccggatc cggagccctc cgtttcctcc 120

ccggagagct ggaccttggg tcacaccccc cagcctgcac ctaaggtgcc cctgtcttcc 180
tccaaccaca tgccccagca acctggggac cctatgggga aaatgtcgct ctatggggct 240
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ccatctctct gctaggctgg ccctggaagg ccactctgct gtccccagag ctctcagccc 420
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agagaggagg tccttcagga caggggctca ggccccaggg cttgggacga ccagcactcc 540
tggcagagag ctctaatttc tgcttccgaa atgggtgtgg accggggttg gggtgggggg 600
gtctctgggc aagaagggtc cctcaagggc tggagctgca aatgtgcccc ctcccaggga 660
gtagagctgt agcctcatgt cttctaatgg ggtgttatga gctggggatg ttaaggtagg 720
ggtgaggggc agtgccatgc tagaggtgct cactgcatcc ttgggcctcc atcaaccatg 780
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agtctcaccc ctggtcagtt tctgcaggag cctccgccac ccaccctgga gccacagaag 1560
gcccagaagc caaatggaca gctggtgaac cccaacaact tctggaagaa cccgaaagat 1620
gtggctgcgc ccacgccc atg gcc tct cag ggg ccc cag gcc tgg gac gtg 1671
Met Ala Ser Gln Gly Pro Gln Ala Trp Asp Val
1 5 10
acc acc act aac tgc tca gcc aat atc aac ttg acc cac cag ccc tgg 1719

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Thr Thr Thr Asn Cys Ser Ala Asn Ile Asn Leu Thr His Gln Pro Trp
            15                  20                  25
ttc cag gtc ctg gag ccg cag ttc cgg cag ttt ctc ttc tac cgc cac     1767
Phe Gln Val Leu Glu Pro Gln Phe Arg Gln Phe Leu Phe Tyr Arg His
        30                  35                  40
tgc cgc tac ttc ccc atg ctg ctg aac cac ccg gag aag tgc agg ggc     1815
Cys Arg Tyr Phe Pro Met Leu Leu Asn His Pro Glu Lys Cys Arg Gly
    45                  50                  55
gat gtc tac ctg ctg gtg gtt gtc aag tcg gtc atc acg cag cac gac     1863
Asp Val Tyr Leu Leu Val Val Val Lys Ser Val Ile Thr Gln His Asp
60                  65                  70                  75
cgc cgc gag gcc atc cgc cag acc tgg ggc cgc gag cgg cag tcc gcg     1911
Arg Arg Glu Ala Ile Arg Gln Thr Trp Gly Arg Glu Arg Gln Ser Ala
                80                  85                  90
ggt ggg ggc cga ggc gcc gtg cgc acc ctc ttc ctg ctg ggc acg gcc     1959
Gly Gly Gly Arg Gly Ala Val Arg Thr Leu Phe Leu Leu Gly Thr Ala
            95                  100                 105
tcc aag cag gag gag cgc acg cac tac cag cag ctg ctg gcc tac gaa     2007
Ser Lys Gln Glu Glu Arg Thr His Tyr Gln Gln Leu Leu Ala Tyr Glu
        110                 115                 120
gac cgc ctc tac ggc gac atc ctg cag tgg ggc ttt ctc gac acc ttc     2055
Asp Arg Leu Tyr Gly Asp Ile Leu Gln Trp Gly Phe Leu Asp Thr Phe
    125                 130                 135
ttc aac ctg acc ctc aag gag atc cac ttc ctc aag tgg ctg gac atc     2103
Phe Asn Leu Thr Leu Lys Glu Ile His Phe Leu Lys Trp Leu Asp Ile
140                 145                 150                 155
tac tgc ccc cac gtc ccc ttc att ttc aaa ggc gac gat gac gtc ttc     2151
Tyr Cys Pro His Val Pro Phe Ile Phe Lys Gly Asp Asp Asp Val Phe
                160                 165                 170
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gtc aac ccc acc aac ctg cta gaa ttt ctg gct gac cgg cag cca cag 2199
Val Asn Pro Thr Asn Leu Leu Glu Phe Leu Ala Asp Arg Gln Pro Gln
175 180 185
gaa aac ctg ttc gtg ggc gat gtc ctg ca 2228
Glu Asn Leu Phe Val Gly Asp Val Leu
190 195

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<211> 848
<212> DNA
<213> Homo sapiens
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tgg ggc ttt ctc gac acc ttc ttc aac ctg acc ctc aag gag atc cac 96
Trp Gly Phe Leu Asp Thr Phe Phe Asn Leu Thr Leu Lys Glu Ile His
20 25 30
ttc ctc aag tgg ctg gac atc tac tgc ccc cac gtc ccc ttc att ttc 144
Phe Leu Lys Trp Leu Asp Ile Tyr Cys Pro His Val Pro Phe Ile Phe
35 40 45
aaa ggc gac gat gac gtc ttc gtc aac ccc acc aac ctg cta gaa ttt 192
Lys Gly Asp Asp Asp Val Phe Val Asn Pro Thr Asn Leu Leu Glu Phe
50 55 60
ctg gct gac cgg cag cca cag gaa aac ctg ttc gtg ggc gat gtc ctg 240
Leu Ala Asp Arg Gln Pro Gln Glu Asn Leu Phe Val Gly Asp Val Leu
65 70 75 80
cag cac gct cgg ccc att cgc agg aaa gac aac aaa tac tac atc ccg 288
Gln His Ala Arg Pro Ile Arg Arg Lys Asp Asn Lys Tyr Tyr Ile Pro

85 90 95

ggg gcc ctg tac ggc aag gcc agc tat ccg ccg tat gca ggc ggc ggt 336
Gly Ala Leu Tyr Gly Lys Ala Ser Tyr Pro Pro Tyr Ala Gly Gly Gly
100 105 110

ggc ttc ctc atg gcc ggc agc ctg gcc cgg cgc ctg cac cat gcc tgc 384
Gly Phe Leu Met Ala Gly Ser Leu Ala Arg Arg Leu His His Ala Cys
115 120 125

gac acc ctg gag ctc tac ccg atc gac gac gtc ttt ctg ggc atg tgc 432
Asp Thr Leu Glu Leu Tyr Pro Ile Asp Asp Val Phe Leu Gly Met Cys
130 135 140

ctg gag gtg ctg ggc gtg cag ccc acg gcc cac gag ggc ttc aag act 480
Leu Glu Val Leu Gly Val Gln Pro Thr Ala His Glu Gly Phe Lys Thr
145 150 155 160

ttc ggc atc tcc cgg aac cgc aac agc cgc atg aac aag gag ccg tgc 528
Phe Gly Ile Ser Arg Asn Arg Asn Ser Arg Met Asn Lys Glu Pro Cys
165 170 175

ttt ttc cgc gcc atg ctc gtg gtg cac aag ctg ctg ccc cct gag ctg 576
Phe Phe Arg Ala Met Leu Val Val His Lys Leu Leu Pro Pro Glu Leu
180 185 190

ctc gcc atg tgg ggg ctg gtg cac agc aat ctc acc tgc tcc cgc aag 624
Leu Ala Met Trp Gly Leu Val His Ser Asn Leu Thr Cys Ser Arg Lys
195 200 205

ctc cag gtg ctc tgaccccagc cgggctacta ggacaggcca gggcacttgc 676
Leu Gln Val Leu
210

tcctgagccc ccatggtatt ggggctggag ccacagtgcc caggcctagc ctttggtccc 736

caaggggagg tggagggttg aggcctacgt gccactgggt gtggtggggt gcaggtagcc 796

agaaagggac ctccctgtgt ggataattct aggaaactga ggcccaggaa cg 848

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<210> 8

<211> 987

<212> DNA

<213> Homo sapiens

<400> 8

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TCA GCC AAT ATC AAC TTG ACC CAC CAG CCC TGG TTC CAG GTC CTG GAG       96
Ser Ala Asn Ile Asn Leu Thr His Gln Pro Trp Phe Gln Val Leu Glu
            20                  25                  30

CCG CAG TTC CGG CAG TTT CTC TTC TAC CGC CAC TGC CGC TAC TTC CCC      144
Pro Gln Phe Arg Gln Phe Leu Phe Tyr Arg His Cys Arg Tyr Phe Pro
        35                  40                  45

ATG CTG CTG AAC CAC CCG GAG AAG TGC AGG GGC GAT GTC TAC CTG CTG      192
Met Leu Leu Asn His Pro Glu Lys Cys Arg Gly Asp Val Tyr Leu Leu
    50                  55                  60

GTG GTT GTC AAG TCG GTC ATC ACG CAG CAC GAC CGC CGC GAG GCC ATC      240
Val Val Val Lys Ser Val Ile Thr Gln His Asp Arg Arg Glu Ala Ile
65                  70                  75                  80

CGC CAG ACC TGG GGC CGC GAG CGG CAG TCC GCG GGT GGG GGC CGA GGC      288
Arg Gln Thr Trp Gly Arg Glu Arg Gln Ser Ala Gly Gly Gly Arg Gly
                85                  90                  95

GCC GTG CGC ACC CTC TTC CTG CTG GGC ACG GCC TCC AAG CAG GAG GAG      336
Ala Val Arg Thr Leu Phe Leu Leu Gly Thr Ala Ser Lys Gln Glu Glu
            100                 105                 110

CGC ACG CAC TAC CAG CAG CTG CTG GCC TAC GAA GAC CGC CTC TAC GGC      384
Arg Thr His Tyr Gln Gln Leu Leu Ala Tyr Glu Asp Arg Leu Tyr Gly
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        115                 120                 125
GAC ATC CTG CAG TGG GGC TTT CTC GAC ACC TTC TTC AAC CTG ACC CTC      432
Asp Ile Leu Gln Trp Gly Phe Leu Asp Thr Phe Phe Asn Leu Thr Leu
    130                 135                 140
AAG GAG ATC CAC TTC CTC AAG TGG CTG GAC ATC TAC TGC CCC CAC GTC      480
Lys Glu Ile His Phe Leu Lys Trp Leu Asp Ile Tyr Cys Pro His Val
145                 150                 155                 160
CCC TTC ATT TTC AAA GGC GAC GAT GAC GTC TTC GTC AAC CCC ACC AAC      528
Pro Phe Ile Phe Lys Gly Asp Asp Asp Val Phe Val Asn Pro Thr Asn
                165                 170                 175
CTG CTA GAA TTT CTG GCT GAC CGG CAG CCA CAG GAA AAC CTG TTC GTG      576
Leu Leu Glu Phe Leu Ala Asp Arg Gln Pro Gln Glu Asn Leu Phe Val
            180                 185                 190
GGC GAT GTC CTG CAG CAC GCT CGG CCC ATT CGC AGG AAA GAC AAC AAA      624
Gly Asp Val Leu Gln His Ala Arg Pro Ile Arg Arg Lys Asp Asn Lys
        195                 200                 205
TAC TAC ATC CCG GGG GCC CTG TAC GGC AAG GCC AGC TAT CCG CCG TAT      672
Tyr Tyr Ile Pro Gly Ala Leu Tyr Gly Lys Ala Ser Tyr Pro Pro Tyr
    210                 215                 220
GCA GGC GGC GGT GGC TTC CTC ATG GCC GGC AGC CTG GCC CGG CGC CTG      720
Ala Gly Gly Gly Gly Phe Leu Met Ala Gly Ser Leu Ala Arg Arg Leu
225                 230                 235                 240
CAC CAT GCC TGC GAC ACC CTG GAG CTC TAC CCG ATC GAC GAC GTC TTT      768
His His Ala Cys Asp Thr Leu Glu Leu Tyr Pro Ile Asp Asp Val Phe
                245                 250                 255
CTG GGC ATG TGC CTG GAG GTG CTG GGC GTG CAG CCC ACG GCC CAC GAG      816
Leu Gly Met Cys Leu Glu Val Leu Gly Val Gln Pro Thr Ala His Glu
            260                 265                 270
GGC TTC AAG ACT TTC GGC ATC TCC CGG AAC CGC AAC AGC CGC ATG AAC      864
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Gly Phe Lys Thr Phe Gly Ile Ser Arg Asn Arg Asn Ser Arg Met Asn
275 280 285

AAG GAG CCG TGC TTT TTC CGC GCC ATG CTC GTG GTG CAC AAG CTG CTG 912
Lys Glu Pro Cys Phe Phe Arg Ala Met Leu Val Val His Lys Leu Leu
290 295 300

CCC CCT GAG CTG CTC GCC ATG TGG GGG CTG GTG CAC AGC AAT CTC ACC 960
Pro Pro Glu Leu Leu Ala Met Trp Gly Leu Val His Ser Asn Leu Thr
305 310 315 320

TGC TCC CGC AAG CTC CAG GTG CTC TGA 987
Cys Ser Arg Lys Leu Gln Val Leu
325

【００６６】

<210> 9
<211> 401
<212> PRT
<213> Homo sapiens
<400> 9

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20 25 30
Gln Phe Leu Gln Glu Pro Pro Pro Pro Thr Leu Glu Pro Gln Lys Ala
35 40 45
Gln Lys Pro Asn Gly Gln Leu Val Asn Pro Asn Asn Phe Trp Lys Asn
50 55 60
Pro Lys Asp Val Ala Ala Pro Thr Pro Met Ala Ser Gln Gly Pro Gln
65 70 75 80
Ala Trp Asp Val Thr Thr Thr Asn Cys Ser Ala Asn Ile Asn Leu Thr
85 90 95

His Gln Pro Trp Phe Gln Val Leu Glu Pro Gln Phe Arg Gln Phe Leu
100 105 110
Phe Tyr Arg His Cys Arg Tyr Phe Pro Met Leu Leu Asn His Pro Glu
115 120 125
Lys Cys Arg Gly Asp Val Tyr Leu Leu Val Val Val Lys Ser Val Ile
130 135 140
Thr Gln His Asp Arg Arg Glu Ala Ile Arg Gln Thr Trp Gly Arg Glu
145 150 155 160
Arg Gln Ser Ala Gly Gly Gly Arg Gly Ala Val Arg Thr Leu Phe Leu
165 170 175
Leu Gly Thr Ala Ser Lys Gln Glu Glu Arg Thr His Tyr Gln Gln Leu
180 185 190
Leu Ala Tyr Glu Asp Arg Leu Tyr Gly Asp Ile Leu Gln Trp Gly Phe
195 200 205
Leu Asp Thr Phe Phe Asn Leu Thr Leu Lys Glu Ile His Phe Leu Lys
210 215 220
Trp Leu Asp Ile Tyr Cys Pro His Val Pro Phe Ile Phe Lys Gly Asp
225 230 235 240
Asp Asp Val Phe Val Asn Pro Thr Asn Leu Leu Glu Phe Leu Ala Asp
245 250 255
Arg Gln Pro Gln Glu Asn Leu Phe Val Gly Asp Val Leu Gln His Ala
260 265 270
Arg Pro Ile Arg Arg Lys Asp Asn Lys Tyr Tyr Ile Pro Gly Ala Leu
275 280 285
Tyr Gly Lys Ala Ser Tyr Pro Pro Tyr Ala Gly Gly Gly Gly Phe Leu
290 295 300
Met Ala Gly Ser Leu Ala Arg Arg Leu His His Ala Cys Asp Thr Leu
305 310 315 320
Glu Leu Tyr Pro Ile Asp Asp Val Phe Leu Gly Met Cys Leu Glu Val

325 330 335

Leu Gly Val Gln Pro Thr Ala His Glu Gly Phe Lys Thr Phe Gly Ile

340 345 350

Ser Arg Asn Arg Asn Ser Arg Met Asn Lys Glu Pro Cys Phe Phe Arg

355 360 365

Ala Met Leu Val Val His Lys Leu Leu Pro Pro Glu Leu Leu Ala Met

370 375 380

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385 390 395 400

Leu

【００６７】

<210> 10

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 10

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【００６８】

<210> 11

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 11

gcacatgccc agaaagacgt cgtc 24

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<210> 12

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

<400> 12

cgttcctggg cctcagtttc ctag 24

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<210> 13

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Oligonucleotide primer for PCR

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【Title of Document】 ABSTRACT

【Abstract】

【Object】 To provide an enzyme having an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage and nucleic acid coding for the enzyme.

【Means for Solution】 A protein having the amino acid sequence shown in SEQ ID NO: 1 in SEQUENCE LISTING, or a protein having the same amino acid sequence as shown in SEQ ID NO:1 except that one or more amino acids are substituted or deleted, or that one or more amino acids are inserted or added, which has an activity to transfer *N*-acetylglucosamine to a non-reducing terminal of Galβ1-4Glc or Galβ1-4GlcNAc group through β1,3-linkage was provided.

【Selected Drawing】 None